SUMMARY
Humic acid inhibited the mutagenicity of various mutagens. The inhibitory effect was desmutagenic, heat resistant and increased with an increase of the humic acid molecular weight. Typical monomeric components of humic acids had no desmutagenic effect. The desmutagenic effect of humic acid was caused by adsorption of mutagen, not by decomposition of mutagen. The adsorption activity was largest at its critical micelle concentration and the adsorbed mutagen was released by ultrasonication.

Humic acids exist in natural environment in large amounts and may play an important role for natural purification by adsorption of mutagens.

INTRODUCTION
River water contains various humic substances and there is a possibility that the humic substances influence on the mutagenicity of mutagens in water. We investigated the inhibitory effect of humic acid on the activity of mutagens.

METHODS
Leaf mould was extracted with 0.5 N NaOH. The pH of the extract was adjusted to 1.0 by HCl and centrifuged. The precipitate was freeze-dried, washed with a small amount of distilled water to remove salts, and again freeze-dried (humic acid). The supernatant was dialyzed and freeze-dried (fulvic acid). 400 g of mould was extracted twice with each 3 - 4 l of distilled water for a day and freeze-dried (water-soluble humic matter).

The decrease of mutagen-induced revertants was investigated by the Ames test (Salmonella typhimurium TA100 and 98). Survival colonies were investigated with B-2 medium and the revertants per survivors were calculated.

RESULTS AND DISCUSSION
(i) Inhibitory effect of humic acid on the mutagenicities of various mutagens and spontaneous mutation
The mutagenicities of B[a]P (Fig. 1) and 2-aminoanthracene with metabolic activation were decreased by humic acid. While, the mutagenicities of 4-nitroquinoline 1-oxide, furyluramid and N-methyl-N'-nitro-N-nitrosoguanidine, which...
Fig. 1. Inhibitory effect of humic acid on the mutagenicity of B[a]P (10 µg/plate). Dotted line indicates spontaneous mutation.

need no metabolic activation for manifestation of the mutagenicity, were not decreased. 2-Nitrofluorene and 1-nitropyrene, not requiring metabolic activation as well, however, the inhibitory effect was observed for. The humic acid itself had no mutagenicity and did not inhibit spontaneous mutation.

(ii) Antimutagen and desmutagen test

Substances which inhibit mutagenicity can be classified into antimutagens and desmutagens. Antimutagens block the process of changing normal cells to mutants. While, desmutagens act on mutagens directly before the mutagens act on cells (ref. 1). As antimutagen test, S. typhimurium was treated with B[a]P and the treated bacteria were washed with phosphate buffer. Ames test was performed with or without humic acid on the treated bacteria and the recovery of damage was investigated. As desmutagen test, B[a]P and humic acid was incubated (37°C, 1 hr) and the treated B[a]P was extracted with ethyl acetate. After ethyl acetate was evaporated, the sample was redissolved in DMSO, tested by Ames test and the decrease of mutagenicity of B[a]P was investigated. Humic acid acts as desmutagen, not as antimutagen (Fig. 2).

(iii) Heat resistance

The desmutagenic activity of humic acid was not decreased by autoclaving (120°C, 15 min), excluding that heat-sensitive substances such as enzymes caused desmutagenicity.
Antimutagen test

Non-treated bacteria
Treated bacteria + water
Treated bacteria + humic acid

Desmutagen test

Spontaneous mutation
Non-treated mutagen
Mutagen treated with water
Mutagen treated with humic acid

Revertants per 10^6 survivors

Fig. 2. Antimutagenic and desmutagenic effects of humic acid.

(iv) Molecular weight of the causative substance

The humic acid was fractionated according to mol. wt. by ultrafiltration. The desmutagenic effect increased with an increase of mol. wt. (Fig. 3). The fraction with mol. wt. above 300,000 contained particles and formed a suspension. A part of this suspension was centrifuged. The desmutagenic effect of the supernatant was smaller than that of suspension. The desmutagenic ability of humic acid may be due to binding of the mutagens to soluble components and small particles.

(v) Desmutagenic effect by humic acid components and related substance

The desmutagenic effect of resorcinol, vanillin, vanillic acid, ferulic acid, protocatechuic acid and benzoic acid, which are constituents of humic acid, were investigated and no effect was observed. On the other hand, lignin, which is a high mol. wt. component of plants, had desmutagenic effect. Therefore, high mol. wt. structure might be necessary for the desmutagenic activity of humic acid.
Fig. 3. Inhibitory effect of various mol. wt. fractions of humic acid on the mutagenicity of B[a]P (10 µg/plate).

Fig. 4. Inhibitory effect of ozone-treated humic acid on the mutagenicity of B[a]P (10 µg/plate). Dotted line indicates spontaneous mutation.

(vi) Decrease of inhibitory effect of ozone-treated humic acid on the mutagenicity of B[a]P

The inhibitory effect of ozone-treated humic acid on the mutagenicity of B[a]P decreased with an increase of ozone treatment time, and it corresponded to
Fig. 5. Decrease of B[a]P by humic acid treatment.

the decreased amounts of KMN04 consumption by the ozone-treated humic acid (Fig. 4).

(vii) Decrease of B[a]P treated by humic acid

B[a]P was treated by humic acid and extracted with ethyl acetate, and investigated by gas chromatography. Only the peaks of solvent and B[a]P were detected and no peaks of decomposition products were observed. It can be concluded that humic acid did not decompose B[a]P.

B[a]P was treated by various amounts of humic acid and extracted by ethyl acetate, and the amounts of B[a]P in the extracts were investigated (Fig. 5). The amount of extracted B[a]P became a minimal value when B[a]P was treated by 10 - 40 mg/2 ml of humic acid as KMN04 consumption.

It is known that humic acid or fulvic acid aggregate to produce micelles at 1 - 10 g/l (ref. 2). This is the same range as found by us for maximal adsorption ability. The aggregation concentration of humic acid corresponds to its critical micelle concentration (CMC). At higher concentrations than CMC, humic acid may form a micelle with hydrophobic parts orientated toward the inside and hydrophilic parts orientated outwards; thus the contribution of the hydrophobic parts for adsorption would decrease, and B[a]P would become more difficult to adsorb to humic acid.

After B[a]P was treated with humic acid and extracted with ethyl acetate, more ethyl acetate was added to the remaining water layer and treated by ultrasonication. The amount of B[a]P in the ethyl acetate extract was investigated
Fig. 6. Relation of ultrasonication time and the release of B[a]P.

(Fig. 6). After two times 20 min ultrasonication, almost all of adsorbed B[a]P was released from humic acid. By this result, it is clear that B[a]P was not decomposed by humic acid, but only adsorbed to humic acid.

(viii) Inhibitory effect of fulvic acid and water-soluble humic matter on the mutagenicity of B[a]P

The fulvic acid and the water-soluble humic matter inhibited the mutagenicity of B[a]P a little.

References